Structural and functional analysis of the Bacillus anthracis nutrient germinant receptor proteins

by

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DEDICATION

To my grandmothers, Mary Koudelka and Faye Jeanell Wilson.

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ABSTRACT

Bacterial spores are dormant, highly resistant cell types formed in response to nutrient deprivation and environmental stressors. When conditions are suitable for growth, spores can quickly convert back to actively replicating, vegetative cells through the process of germination. Germination is a required step in the infectious cycle of many spore-forming pathogens, including *Bacillus anthracis*, the etiologic agent of anthrax. Nutrient germinant receptors (nGRs) are packaged within the spore and initiate germination in response to specific nutrient molecules. The nGRs are comprised of three subunits: the A-, B-, and C-type proteins. Currently, the exact mechanism of action of these proteins is unknown. Therefore, the goal of this work was to improve our understanding of the structure of the nGR complex, and the role of the individual nGR proteins in triggering germination to provide insight into the overall mechanisms of germination initiation. Here, the first successful overexpression of the A- and B-type nGR proteins is described. Using this system, the membrane topology of the $GerH_A$, $GerH_B$ and $GerH_C$ proteins was determined and the data were used to create a model of the nGR protein topology in the inner membrane of the spore. Importantly, these data represent the first structural information of the A- and B-type nGR subunits and have provided insight into their roles in triggering

germination. In addition, it is demonstrated that the A-type subunits of the *B. anthracis* nGRs share some functional redundancy, influence ligand specificity, and may require co-expression with the B- and C-type proteins for proper function. This dissertation work has advanced our understanding of the structure and function of the individual nGR subunits, an important step toward determining the mechanism of action of these receptors. This information may lead to identification of novel decontamination methods for preventing infections by the spore-forming pathogens.